ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

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CONTENTS

	Page
Summary	1
Introduction	2
Experimental	2
Dry Heat Resistance of Spores in and on Various Materials	2
Relationship of Water Activity to Dry Heat Inactivation of Spores Encapsulated In Lucite	4
Projected Research for Ninth Quarter	5
References	6
Table 1	7
Table 2	8
Table 3	9
Table 4	10

SUMMARY

The dry heat resistance of Bacillus subtilis var. niger spores in and on various materials was determined. D values for spores encapsulated in epoxy and exposed to temperatures of 115, 125, and 135° C were found to be respectively: D_{115} = 16.1 hours, D_{125} = 5.3 hours, D_{135} = 1.9 hours. The z_D value for spores in epoxy was 21.4 Centigrade degrees with a 95% C.I. of 20.8 to 22.1 Centigrade degrees. D values for spores trapped between stainless steel surfaces mated at 12 and 150 inch-pounds and exposed to 135° C were found to range respectively: D_{135} for 12 inch-pounds = 7.9 to 8.1 minutes and D_{135} for 150 inch-pounds = 22.0 to 25.5 minutes. Inoculated Lucite powders that were stored for two weeks at relative humidities of 20, 40, 60, and 80%, and then formed into rods and exposed to 135° C were found to have D values of 73.5, 88.7, 67.4, and 36.0 minutes, respectively.

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INTRODUCTION

Efforts during the Eighth Quarter were expended to establish the dry heat resistance of microbial spores in and on various materials and to determine the effect of water activity on the dry heat resistance of encapsulated spores.

EXPERIMENTAL

Dry Heat Resistance of Spores in and on Various Materials

A method for the inoculation and recovery of viable spores from epoxy resin was described in the Seventh Quarterly Report of Progress; methods for paper and Lucite were described in the Fourth and Fifth Quarterly Report of Progress, respectively. A modification of the procedure reported in the Seventh Quarterly Report of Progress for determining the dry heat resistance of spores trapped between mated stainless steel surfaces was devised to eliminate the possibility of moisture adsorption by the stainless steel disks during assembly of the piece parts. Data on the dry heat resistance of spores trapped between mated steel surfaces were collected by the procedure described below.

Stainless steel washers (#302) with a No. 4 finish were inoculated and dried at 50° C for 1 hours, as described previously. Dry, inoculated

washers were mounted on the male lug with the inoculated surface adjacent to the uninoculated No. 4 finish of a second washer. Male and female lugs were loosely threaded together, placed in TDT tubes, and the tubes and torque wrench were sealed in a polyethylene bag containing silica gel. The flexible desiccator was stored overnight at 20° C. Working from outside of the plastic bag, the units were torqued the desired amount, the bag was opened, and a constriction was made in the TDT tubes. Silica gel was placed in the upper well of the tubes, and the tubes were stored in a desiccator over silica gel overnight at 20° C, after which the tubes were sealed at the constriction and exposed to the test temperature in a silicone bath. Heated tubes were cooled, washed, surface disinfected, and opened as described previously. Both washers were placed in a test tube (16 x 150mm) containing 5 milliliters of phosphate buffered dilution water. The tubes were placed in an ultrasonic (Branson A-300) bath for 10 minutes, and a dilution plate count of the liquid contents was made as previously described.

D values and the corresponding 95% confidence intervals for Bacillus subtilis var. niger spores in and on various materials exposed to a dry heat temperature of 135° C are shown in Figure 1 and Table 1. It is evident from the data that encapsulated spores exhibit a greater resistance to dry heat inactivation than spores located on or between surfaces. Spores located between surfaces torqued at 150 inch-pounds are more resistant to dry heat than spores subjected to 12 inch-pounds of torque.

Dry heat decimal reduction time curves for Bacillus subtilis var. niger spores encapsulated in epoxy resin and Lucite are shown in Figure 2.

D values for Lucite from 105° C to 160° C have been reported previously and are summarized in Table 2. Table 3 shows D values and 95% confidence intervals obtained for spores encapsulated in epoxy resin at 115, 125, and 135° C for duplicate experiments. The z_D value for epoxy was calculated from a linear regression of the semilogarithmic plot of D values and their corresponding temperatures, and was found to be equal to 21.4 Centigrade degrees with a 95% Confidence Interval of 20.8 to 22.1 Centigrade degrees.

Relationship of Water Activity to Dry Heat Inactivation of Spores Encapsulated in Lucite

In view of the results obtained by Murrell and Scott (4) indicating that the water activity of spores profoundly influences dry heat inactivation, inoculated Lucite powder was stored for 2 weeks at 25° C in a sealed container over various saturated salt solutions to obtain the following Relative Humidity: 20, 40, 60, and 80%. Assuming that equilibrium occurred, these conditions would result in spore water activities of 0.2, 0.4, 0.6, and 0.8. The equilibrated powders were prepared and formed into rods according to the method described in the Seventh Quarterly Report of Progress and D values were obtained at 135° C (Table 4, and Figure 3). These data corroborate the findings of Murrell and Scott, namely, that spores of low and high water activity have lower D values than spores of intermediate water activity. In spite of the differences in systems employed between the two laboratories (naked spores for Murrell and Scott versus encapsulated spores), the influence of moisture on spore survival during exposure to dry heat is marked.

The apparent relationship between spore resistance and the material in or on which the spore is located may involve water activity. Diffusion of water from the spore or from the micro-environment surrounding the spore may be prevented by encapsulation in inert material such as Lucite and epoxy or by entrapment between surfaces under heavy torque, thus permitting maintenance of intra-cellular water activities and/or micro-environmental Relative Humidity favorable for survival. Spores impregnated in paper or located between loosely mated surfaces or on exposed surfaces may be expected by diffusion to readily lose moisture from their surfaces to the surrounding hot air and attain internal moisture levels below those affording protection. This would be particularly true under non-steady state conditions such as in a flowing gas system or in sealed tubes where the gaseous volume is large in comparison to the amount of water given up by the spores.

PROJECTED RESEARCH FOR THE NINTH QUARTER

An effort will be made to further establish the role of water activity in the dry heat sterilization process. Data will be collected on the dry heat resistance of spores adjusted to very low and high water activities in Lucite. The effect of water activity on the dry heat resistance of spores located on various surfaces will also be studied.

REFERENCES

1. Fourth Quarterly Report of Progress, January - March 31, 1966, NASA Research Project R-36-015-001.
2. Fifth Quarterly Report of Progress, April - June 30, 1966, NASA Research Project R-36-015-001.
3. Seventh Quarterly Report of Progress, October - December 31, 1966, NASA Research Project R-36-015-001.
4. Murrell, W. G. and Scott, W. J. The heat resistance of bacterial spores at various water activities. J. Gen. Microbiol. 43:411-425, 1966.

TABLE 1

Inactivation of Bacillus subtilis var. niger Spores*
In or On Various Materials Exposed to a Dry-Heat
Temperature of 135° C

Material in or on which spores located	D 135° C	95% C.I.**
Scotchcast epoxy (electrical resin #5) [spores encapsulated in rods]	1.9 hours 1.9 hours	1.7 to 2.1 hours 1.7 to 2.1 hours
Lucite [spores encapsulated in rods]	1.4 hours 1.3 hours	1.3 to 1.5 hours 1.2 to 1.4 hours
Stainless steel washers (302 #4) mated at 150 inch-pounds of torque [spores at interface]	25.5 min. 22.0 min.	20.7 to 33.3 min. 18.2 to 27.7 min.
Filter paper strips (1" x 3/8" Whatman #2) [spores impregnated]	16.1 min. 17.2 min.	15.3 to 16.9 min. 16.0 to 18.3 min.
Stainless steel washers (302 #4) mated at 12 inch-pounds of torque [spores at interface]	8.1 min. 7.9 min.	7.5 to 8.8 min. 6.4 to 10.3 min.
Stainless steel strips (302 #4 - 1" x 1/8") [spores on surface]	2.6 min. 2.9 min.	2.2 to 3.0 min. 2.3 to 3.8 min.

* Inoculum = 1×10^7 to 1×10^8 per gram or per surface

** 95% Confidence Interval

TABLE 2

D Values for *Bacillus subtilis* var. *niger* Spores*

Encapsulated in Lucite Rods

Dry-Heat Exposure Temperature °C	D Value	95% Confidence Interval
105	32.0 hours	26.4 to 33.6 hours
	28.8 hours	26.4 to 36.0 hours
115	15.6 hours	13.4 to 17.8 hours
	15.1 hours	13.7 to 16.2 hours
120	6.2 hours	5.6 to 6.7 hours
	5.9 hours	5.0 to 6.8 hours
125	3.1 hours	2.5 to 3.6 hours
	3.4 hours	2.9 to 3.9 hours
135	1.4 hours	1.3 to 1.5 hours
	1.3 hours	1.2 to 1.4 hours
160	4.6 minutes	4.4 to 4.8 minutes
	4.1 minutes	3.6 to 4.5 minutes

* Inoculum = 1×10^8 per gram

TABLE 3

D Values for *Bacillus subtilis* var. *niger* Spores*

Encapsulated in Epoxy Rods

Dry-Heat Exposure Temperature °C	D Value (Hours)	95% Confidence Interval (Hours)
115	16.1	15.2 to 17.2
	16.1	15.2 to 16.8
125	5.3	5.1 to 5.7
	5.3	5.0 to 5.6
135	1.9	1.7 to 2.1
	1.9	1.7 to 2.1

* Inoculum = 1×10^8 spores per gram

TABLE 4

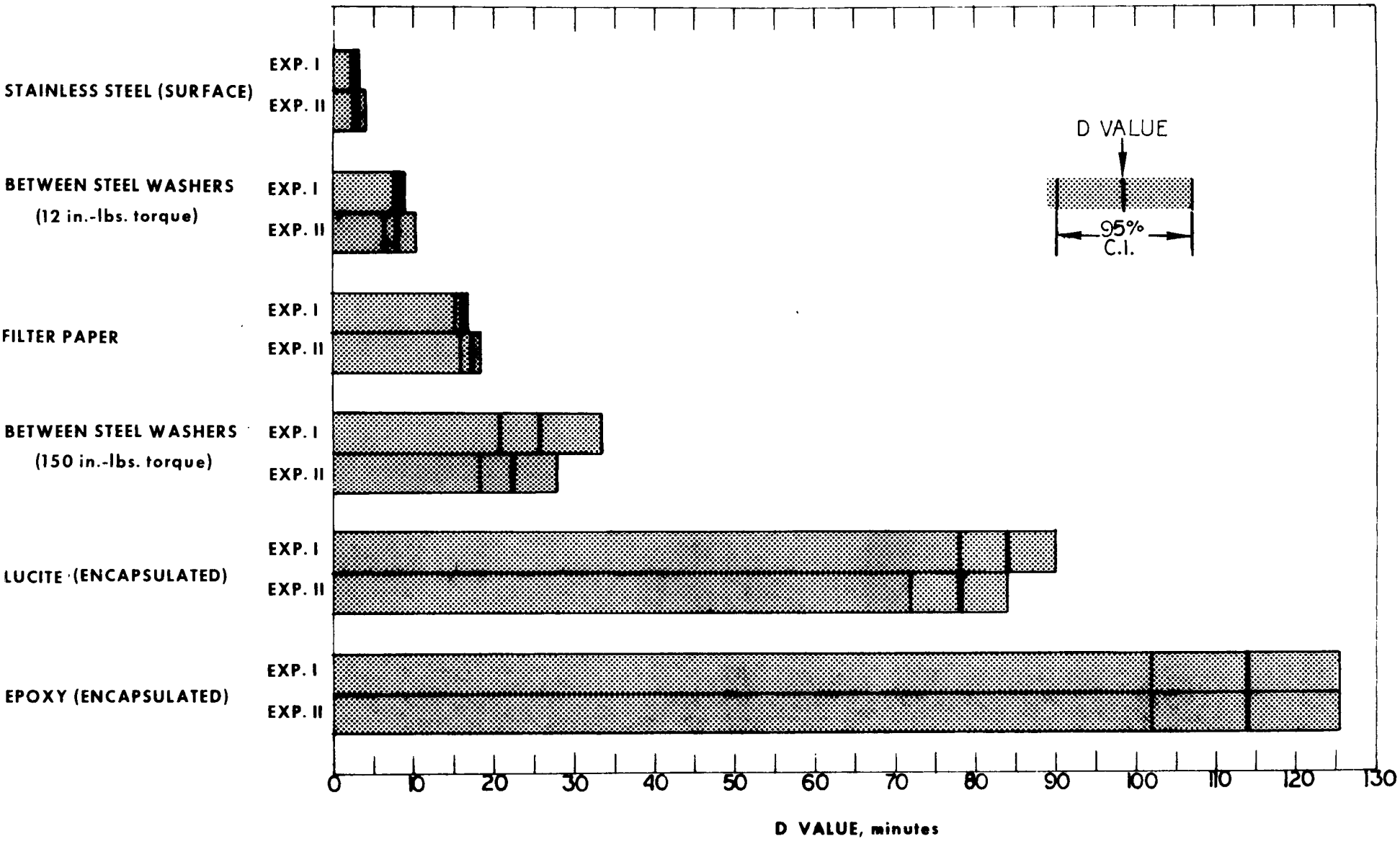
Influence of Water Activity on Inactivation of *Bacillus subtilis* var. *niger* Spores* Encapsulated in Lucite and Exposed to Dry Heat

Water Activity a_w	D 135° C (Minutes)	95% Confidence Interval (Minutes)
0.2	73.5	67.5 to 80.5
0.4	88.7	83.9 to 94.1
0.6	67.4	60.6 to 75.9
0.8	36.0	33.1 to 39.2
0.9	3 to 5**	----

* Inoculum = 1×10^8 spores per gram

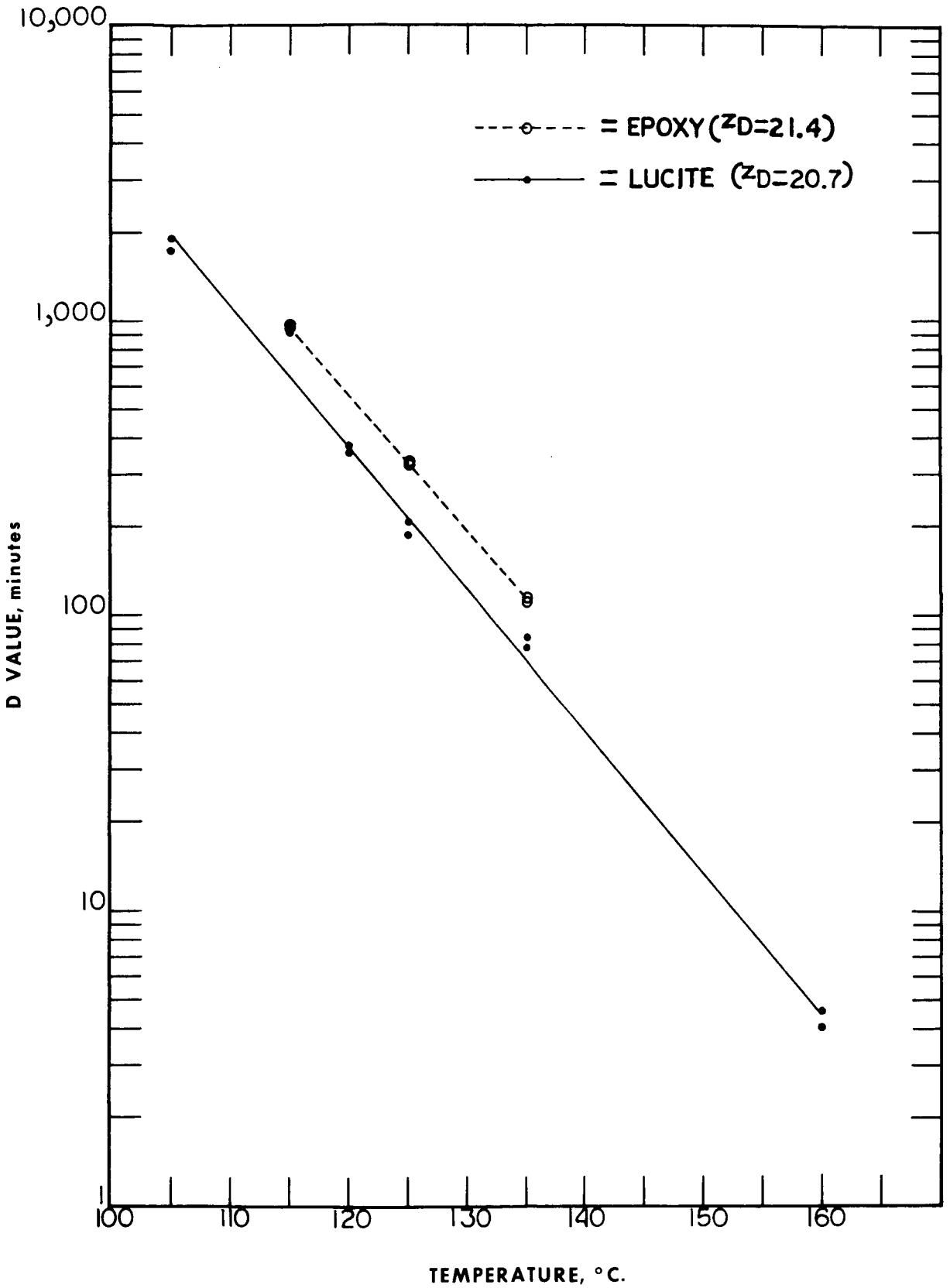
** Preliminary data

FIGURE 1



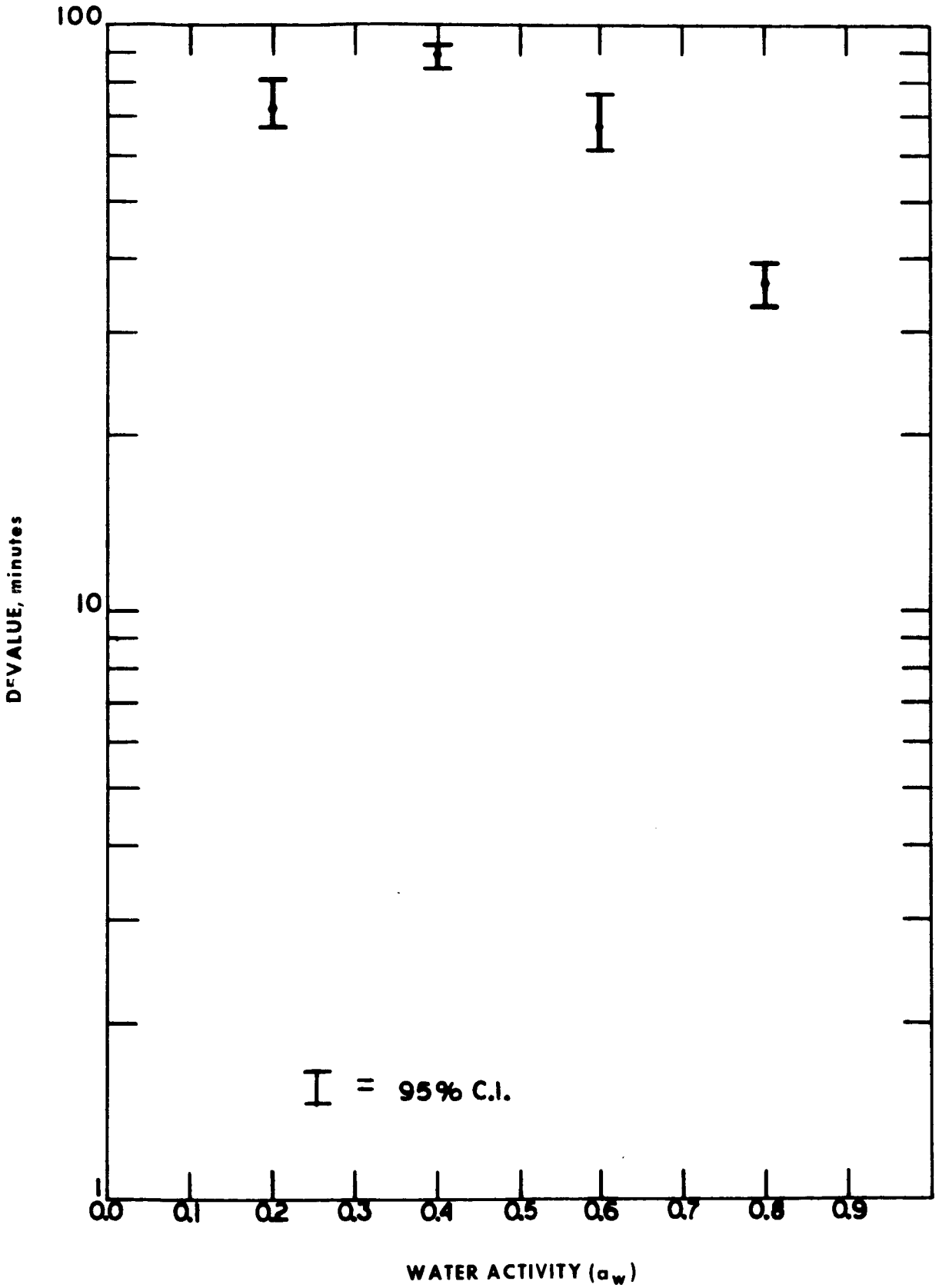
INACTIVATION OF Bacillus subtilis var. niger SPORES IN OR ON VARIOUS MATERIALS EXPOSED TO A DRY HEAT TEMPERATURE OF 135°C.

FIGURE 2



DRY HEAT DECIMAL REDUCTION TIME CURVES FOR
Bacillus subtilis var. niger SPORES

FIGURE 3



INFLUENCE OF WATER ACTIVITY ON DRY HEAT RESISTANCE OF Bacillus subtilis var. niger SPORES ENCAPSULATED IN LUCITE